

PLK1 Kinase Assay

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Scientific Background:

PLK1 is a member of the Polo-Like Kinase family that localizes to centrosomes or spindle pole bodies and undergoes dramatic subcellular relocation during the cell cycle. Deregulated activities of PLKs often result in abnormalities in centrosome duplication, maturation, and/or microtubule dynamics (1). PLKs also regulate the function of the Golgi complex. Deregulated expression of human PLK1 is strongly correlated with the development of many types of malignancies, and ectopic expression of PLK1 dominant negative protein leads to rapid cell death (2).

1. Nigg, EA. et al: Dynamic changes in nuclear architecture during mitosis: on the role of protein phosphorylation in spindle assembly and chromosome segregation. *Exp Cell Res.* 1996 Dec 15; 229.
2. Dai, W. et al: Polo-like kinases and the microtubule organization center: targets for cancer therapies. *Prog Cell Cycle Res.* 2003; 5:327-34.

ADP-Glo™ Kinase Assay

Description

ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.

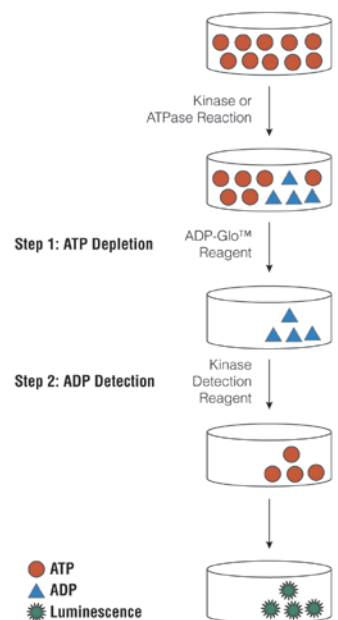


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

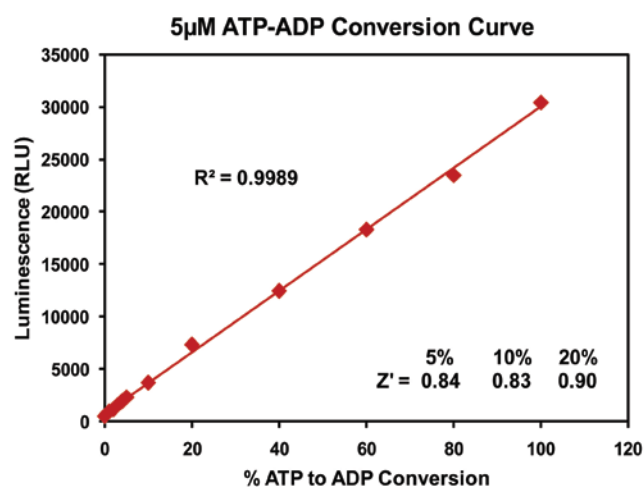


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
 - 1 μ l of inhibitor or (5% DMSO)
 - 2 μ l of enzyme (defined from table 1)
 - 2 μ l of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 μ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. PLK1 Enzyme Titration. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

PLK1, ng	100	50	25	12.5	6.3	3.1	1.6	0
RLU	3885	3777	3079	2753	2274	1979	1383	979
S/B	4	4	3.1	2.8	2.3	2.0	1.4	1
% Conversion	11	11	7.9	6.5	4.5	3.3	0.8	0

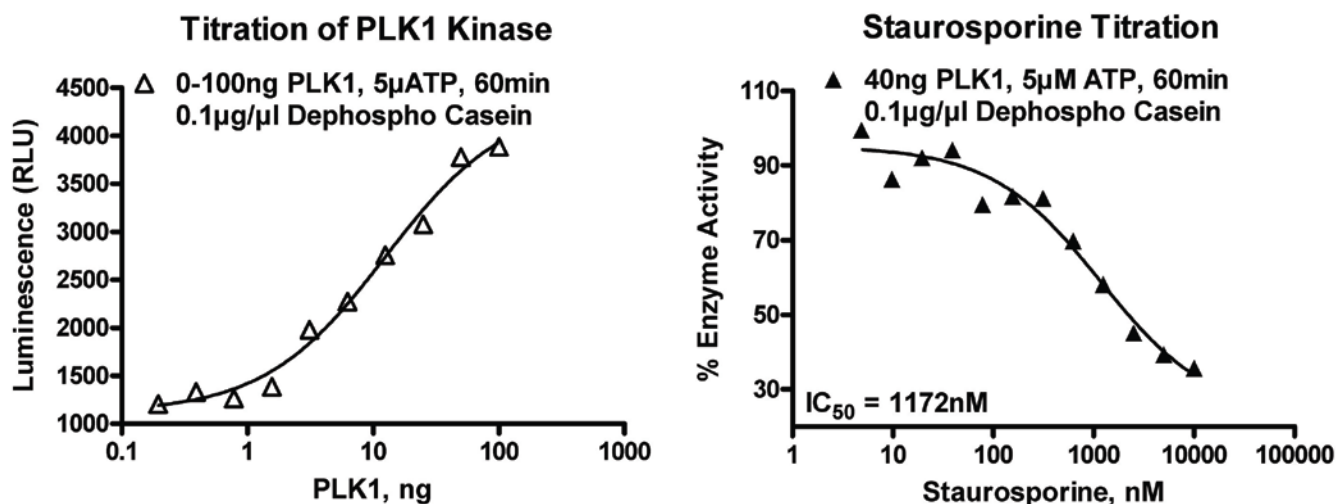


Figure 3. PLK1 Kinase Assay Development. (A) PLK1 enzyme was titrated using 5 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 40ng of PLK1 to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PLK1 Kinase Enzyme System	Promega	V2841
ADP-Glo™ + PLK1 Kinase Enzyme System	Promega	V8041

PLK1 Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 50 μ M DTT.